

Studies on the Association between the Anticardiolipin Antibody and Preeclampsia

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Summary. In order to evaluate the implication of autoimmune mechanisms in the genesis of preeclampsia, two studies were comprised this work. The one is the assessment of the prevalence of the anticardiolipin antibody in patients with severe preeclampsia. The other is the clinical trial of the prophylaxis of preeclampsia in patients experiencing severe preeclampsia and/or intrauterine fetal growth retardation (IUGR). The positive rate of anticardiolipin antibody in cases with severe preeclampsia was significantly higher than that in normotensive pregnant women. The medication, which consisted of low doses aspirin and a traditional Chinese herbal medicine Sairei-To (Chan-Ling Tang)—which is reported to have glucocorticoid-like effect, was administered to 7 patients who had experienced severe preeclampsia with positive anticardiolipin antibody since the early stage of their new pregnancy. In one case glucocorticoid was also administered. Of seven patients, 5 patients delivered normal sized infants without preeclampsia with the improvement of autoimmune abnormalities. Undersized infants were delivered accompanied by manifestations of preeclampsia in the remaining 2 patients in whom significantly high titers of anticardiolipin antibody continued during their prenatal course. These data indicate that preeclampsia is one of the clinical entities of reproductive autoimmune failure syndrome (RAFS), and that the prediction and prophylaxis of preeclampsia might be possible from the viewpoint of autoimmunity.

INTRODUCTION

Preeclampsia is a form of hypertension that is unique to human pregnancy, the etiology of which is almost unclear. Recently, the concept of reproductive autoimmune failure syndrome was proposed,¹⁾ and autoimmune factors such as lupus anticoagulant and anticardiolipin antibody have been considered to be associated with the manifestation of preeclampsia.²⁻⁴⁾

It is well known that fetal wastage, preeclampsia and intrauterine growth retardation (IUGR) are major complications for pregnant women with systemic lupus erythematosus (SLE).⁵⁾ On the other hand, antiphospholipid antibodies may be responsible for repeated early miscarriage,^{6,7)} and the efficacy of treatment for these patients using prednisolone and low doses aspirin has been reported.⁸⁻¹⁰⁾ Against this context, it was considered worthwhile to investigate the existence of autoimmune abnormality in patients with preeclampsia and to pursue the possibility of prediction and prophylaxis of preeclampsia from the viewpoint of autoimmunity. The purpose of this study was to evaluate the prevalence of anticardiolipin antibody in patients with preeclampsia and also to evaluate the possibility of prophylaxis of preeclampsia from the viewpoint of autoimmunity.

MATERIALS AND METHODS

I. Assessment of anticardiolipin antibody

Study population: Serum samples from 50 patients with severe preeclampsia were used for examination of the anticardiolipin antibody. Severe preeclampsia was diagnosed according to the clinical criteria defined by the "International Society for the Study of Hypertension in Pregnancy."¹¹⁾ The serum samples from 50 normotensive pregnant women who were age and parity matched with patients were used as controls (Table 1). The positive rate of anticardiolipin antibody in the patients' group was compared with that in the control group.

Measurement of anticardiolipin antibody: Anticardiolipin antibody was screened by the modified enzyme-linked immunosorbent assay by Loizou et al.¹²⁾ Microtiter plates were coated with the cardiolipin and blocked for nonspecific binding and augmented

with 5% AB serum for a cofactor. Duplicate serum samples were diluted to 1:50 in phosphate-buffered saline solution with 10% bovine serum albumin and were added onto microtiter plates which were incubated for 60 min. Affinity-purified peroxidase-conjugated goat antihuman immunoglobulin (Sigma Chemical Co., St. Louis, U.S.A.) in phosphate-buffered saline solution with 1% bovine serum albumin were then added. After 60 min of incubation, the plates were washed and o-phenylene diamine and hyperoxide were added as substrate. The plates were read at 492 nm with a spectrophotometer. Control serum samples with elevated anticardiolipin antibody (positive well) as well as a blank well with no serum (negative well) were assayed in parallel with each plate. The ELISA units were calculated firstly according to the formula shown below:

$$\text{ELISA unit} = \frac{\text{sample OD} - \text{negative control OD}}{\text{positive control OD} - \text{negative control OD}} \times 100$$

ELISA unit was determined in 200 normal healthy females, and mean value and standard deviations (SD) were calculated. The titer of anticardiolipin antibody in the positive control serum, which was obtained under informed consent from a patient with recurrent fetal wastages (2 late abortions and 2 IUDFs) and thrombophlebitis, was 167 GPL units according to the

Table 1. Age and parity of patients and control group

	Mean age	Mean parity
Preeclampsia (n=50)	29.6±4.9 y.o.	0.36±0.27
Normal pregnant women (n=50)	28.9±4.3 y.o.	0.41±0.35

comparative study employing the standard serum provided by Harris et al.¹³⁾ Sera which exceeded three SD above the mean value (cut-off value) of ELISA units were assigned as positive, and the cut-off value corresponded to 20 GPL of anticardiolipin antibody.

II. Clinical trial of prophylaxis of preeclampsia

Patients: The obstetric histories of the 7 women in this study are summarized in Table 2. All previous pregnancies had resulted in severe preeclampsia, and 5 cases were complicated with intrauterine growth retardation of the infant. Of 7 infants, two had died during the pre- or postnatal period probably due to their prematurity. Selected laboratory data for each patient are shown in Table 3. Before treatment, all patients had positive anticardiolipin antibody tests. In two patients, significantly elongated activated partial thromboplastin time (APTT, longer than 40 sec determined by the 99% confidence interval in normal healthy individuals) was observed.

Drug therapy and obstetric management: A Chinese traditional herbal medicine Sairei-To (Chan-Ling-Tang) (9.0 g/day) and aspirin (81 mg/day, one tablet of children's aspirin) were administered orally to each patient from the early stage of pregnancy with sufficiently informed consent concerning the therapy. In one patient, Sairei-To was administered before pregnancy and predonisolone was added during pregnancy, because the subject had experienced 8 spontaneous abortions before her tenth pregnancy which had resulted in severe preeclampsia, as we have previously described as a brief report.¹⁴⁾ The drug was discontinued at delivery in all patients. Patients were examined at least twice monthly throughout their pregnancy. In most cases, serial ultrasound

Table 2. Previous obstetric histories and laboratory data of patients

Cases	Age	Parity (Gravida-Para)	Max. B. P. ^a (mmHg)	U. P. ^b (g/day)	G. W. ^c	Outcome of pregnancy		APTT ^d (seconds)	CL ^e (GPL unit)
						Mode	Body weight		
1	26	0-0	200/110	2.6	29w6d	C/S ^f	908g (-1.92SD ^g)	34.2	28.4
2	33	9-0	180/100	0.8	27w3d	C/S	758g (-1.50SD)	42	52.9
3	25	1-0	190/120	3.2	31w3d	C/S	832g (-2.78SD)	27.8	42.2
4	18	0-0	170/110	3	32w5d	C/S	1640g (-0.39SD)	28.4	20.8
5	28	0-0	160/100	4	33w2d	C/S	1412g (-2.94SD)	n.t. ^h	n.t.
6	32	0-0	170/130	3.5	28w2d	C/S	665g (-3.94SD)	n.t.	n.t.
7	30	0-0	160/100	6.2	38w0d	Vaginal	2722g (-0.61SD)	n.t.	n.t.

a, maximum blood pressure; b, urinary protein; c, gestational weeks; d, activated partial thromboplastin time; e, anticardiolipin antibody; f, cesarean section; g, compared with mean body weight of newborns in Japan; h, not tested.

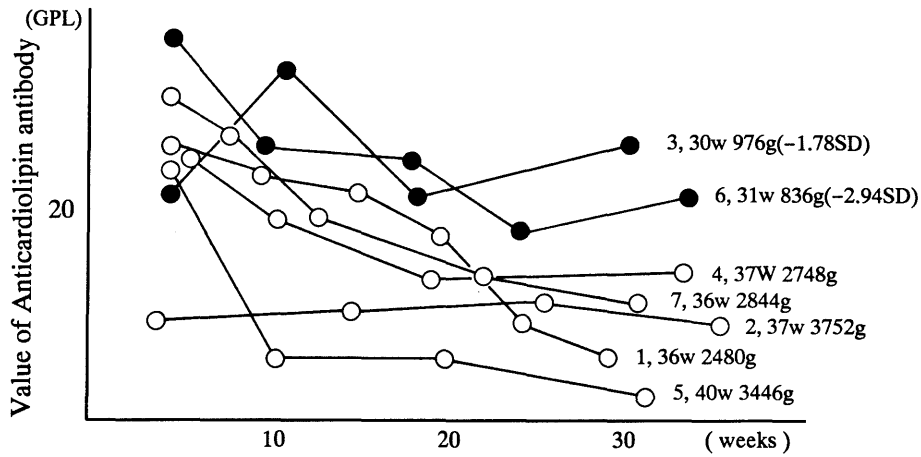


Fig. 1. Dynamic changes of anticardiolipin antibody during the prenatal course in patients with previous preeclampsia and/or IUGR. Vertical axis indicates the titer of anticardiolipin antibody expressed in GPL units. Horizontal axis represents gestational weeks. Open circles represent the patients in whom the titer of anticardiolipin antibody decreased into the normal range, and shaded circles represent the patients in whom the titer of anticardiolipin antibody remained positive during their prenatal course. The case number listed on the Tables and the outcome of current pregnancies, i.e., gestational weeks of delivery, body weight of the infant, etc., are followed by the lines. Two cases (Cases 3 and 6) experienced repeated severe preeclampsia and 5 (Cases 1, 2, 4, 5 and 7) experienced uneventful prenatal courses. Note that the patients in whom the anticardiolipin antibody normalized during the prenatal course delivered infants of normal weight with no manifestation of preeclampsia, and that the patients with positive anticardiolipin antibody resulted in preeclampsia with IUGR.

Table 3. Selected laboratory data of 7 patients at the 1st trimester of current pregnancy

Case	CL ^a (GPL unit)	ANA ^b	APTT ^c (seconds)
1	28.3	160×	28.9
2	11.2	160×	32.0
3	24.3	80×	27.9
4	22.0		25.1
5	26.7		44.0
6	44.2	320×	33.3
7	38.5	320×	42.0

a, anticardiolipin antibody; b, antinuclear antibodies; c, active partial thromboplastin time.

examinations were done from the first trimester onward, and fetal heart rate monitoring was performed each week, beginning at 30 weeks of gestation.

Table 4. Positive rates of anticardiolipin antibody in patients with preeclampsia

	No. of patients	anticardiolipin antibody	positive rate
Preeclampsia	50	12	24.0%
Normal pregnant women	50	2	4.0%

} p < 0.05

RESULTS

I. Positive rate of anticardiolipin antibody (Table 4)

Of 50 patients with severe preeclampsia, anticardiolipin antibody was detected in 12(24.0%). The positive rate of anticardiolipin antibody was significantly higher in patients with severe preeclampsia than that

in 50 normotensive pregnant women.

II. Clinical course and outcome of pregnancy in treated patients

The alteration of anticardiolipin antibody titer during pregnancy and the results of each pregnancy is shown in Fig. 1. In five patients (Cases 1, 2, 4, 5 and 7), a decrease of anticardiolipin antibody titer was observed from the 1st trimester, entering into the normal range (anticardiolipin antibody <20 GPL) in the 2nd trimester. In these patients, the symptoms of preeclampsia were not observed and normal mature infants were delivered at term. On the other hand, in the remaining 2 patients (Cases 3 and 6), anticardiolipin antibody was observed to be positive throughout the prenatal course. In one of the patients (Case 3), placental abruption suddenly occurred accompanied by the elevation of blood pressure, and emergency surgery was performed. The offspring, although exhibiting neonatal asphyxia at birth, survived and recovered with treatment in the neonatal intensive care unit. One other (Case 6) also experienced severe preeclampsia complicated with IUGR and underwent a cesarean section.

DISCUSSION

Recently, the concept of reproductive autoimmune failure syndrome has been suggested.¹⁾ Autoimmune factors such as lupus anticoagulant (LAC) or antiphospholipid antibodies are considered to be implicated in the genesis of unexplained recurrent fetal wastage due to the formation of thrombi intervillous spaces. Carreras et al. reported that the LAC suppressed the production of prostacyclin from endothelial cells of the mouse aorta.¹⁵⁾ They suggested that such a condition might induce the predominance of thromboxane A₂, which has strong vasoconstriction and platelet aggregation, followed by the formation of thrombi. Some authors reported on the effectiveness of immune suppression and anticoagulant therapy for patients with recurrent fetal wastage who were positive for LAC or antiphospholipid antibodies.^{9,10)} We have also reported on the efficacy of the therapy.⁹⁾ Concerning preeclampsia, a relationship between autoimmune factors and the pathophysiology of preeclampsia has been suggested to exist. El-Roeiy et al. demonstrated increased autoantibody levels in hypertensive diseases of pregnancy in comparison with non-pregnant controls.³⁾ Milliez et al. also reported the significantly higher prevalence of autoantibodies

in pregnancies complicated by hypertension as compared with normal pregnant women.⁴⁾ This current study provides further quantitative evidence of the association between antiphospholipid antibodies and the manifestation of preeclampsia, i.e., a significantly high positive rate of anticardiolipin antibody was observed in patients with severe preeclampsia compared with the normotensive pregnant women, although no correlation between the anticardiolipin antibody titer and the severity of preeclampsia was observed (data not shown). Branch et al. reported a high rate of the manifestation of preeclampsia in patients with recurrent fetal wastage who were treated with prednisolone and low doses of aspirin.¹⁰⁾ These data suggest the crucial role of autoimmune factors such as LAC or antiphospholipid antibodies in the genesis of preeclampsia. In this context, we performed prophylactic therapy for 7 patients who had experienced severe typed preeclampsia and/or IUGR and were positive for anticardiolipin antibody. A traditional Chinese herbal medicine Sairei-To (Changling Tang) was used. This was substituted for prednisolone for two main reasons: one being the well-known side effects of prednisolone and the other being that Sairei-To has been reported to possess corticosteroid-like pharmacologic effects. Nine grams of Sairei-To contain 6.0 gram of dried extract of a crude drug mixture containing the ingredients of Bupleurum Root (1.05 gram), Alisma Rhizome (0.75 gram), Pinellia Tuber (1.05 gram), Scutellaria Root (0.45 gram), Atractylodes Lancea Rhizome (0.45 gram), Jujube (0.45 gram), Chuling (0.45 gram), Ginseng (0.45 gram), Hoelen (0.45 gram), Glycyrrhiza (0.30 gram), Cinnamon Bark (0.30 gram) and Ginger (0.15 gram). The saicosaponin contained in Bupleurum Root and the Ninjinsaponin contained in Ginseng are reported to have a corticosteroid-like effect, and Sairei-To is reported to be used for decreasing the dosages of corticosteroid in patients with nephrotic syndrome.¹⁶⁾ In this study, in five out of 7 patients, their new pregnancy continued uneventfully with the improvement of the titer of anticardiolipin antibody, which may suggest the possibility of prophylaxis of preeclampsia induced by the autoimmune mechanism.

Symptoms of preeclampsia are believed to improve after delivery, while the titer of anticardiolipin antibody remains positive, according to our preliminary follow up study. The reason for such a discrepancy might be understood as follows. In general, blood pressure decreases during the prenatal course in spite of plasma volume expansion in normal pregnant women which is thought to be due to vascular refractoriness against angiotensin II.¹⁷⁾ Antiphospholipid

antibodies were observed to suppress the formation of prostacyclin which has a strong vasodilating effect,¹⁵⁾ and it is possible that the existence of antiphospholipid antibodies specifically induces hypertension during pregnancy. Although the correlation between the appearance of antiphospholipid antibodies and the manifestation of preeclampsia is believed to exist on the basis of the results of this study, the efficacy of the treatment using Sairei-To and low doses of aspirin for the prevention of preeclampsia and IUGR cannot be yet determined because of the unavailability of a control group. Moreover, the number of cases entered into the current prophylactic study is considered to be insufficient for obtaining the conclusion. Further case-controlled trials are mandatory for elucidating the efficacy of the prophylactic treatment for patients who have experienced severe preeclampsia and are positive for antiphospholipid antibodies.

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